

CAST
LEAF

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PROGRAM NUMBER : 920108
 PROGRAM NAME : Cast Leaf
 PROGRAM COORD. : G. Gellatly
 WRITTEN BY : G. Gellatly; R. G. Uhl
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: Pilot plant NBL was judged by the Cast Leaf Panel to be sufficiently similar to production RCB in 100% and 24% blend cigarettes to warrant Semiworks production of a Marlboro blend for comparison with RCB. These cigarettes will be available for evaluation in December. BL Plant trials are planned in the first quarter of 1993 for factory qualification of NBL product through POL testing. Preparation of a business plan will be formulated by Engineering by December 1993 to establish feasibility of modernizing the BL Plant to include the NBL process.

NBL sheet weight (110–170 kg/m²) was shown to have no effect on filling power or smoke analysis of 24% MF blends (total recon substitution). This could be an alternative to line speed increase for BL plant capacity increase.

Oxalic acid was shown to be an alternative to DAP as a pectin release agent. The sheet quality was unaffected, but the tar delivery was not reduced as expected with the elimination of DAP. Two families of recon tobacco appear to exist. One is formed by chemical reaction (RCB) which is a homogeneous product with a high tar delivery. The other is formed by physical means which has a more open structure resulting in a lower tar delivery (CL guar).

I. Objective: Optimize the current RCB process for improved sheet physical properties, production capacity, and environmental goals.

A. Strategy: Utilize the Cast Leaf Pilot Plant to develop new technology applicable to the present RCB process to improve sheet quality without changing the subjective character or delivery

1. Results: Pilot plant process conditions were defined to obtain subjective parity of NBL with production RCB. Aging time and dryer profile were determined to be the most important variables affecting subjective character. These conditions were very similar to those in the BL Plant. Difficulty identifying processing conditions for subjective parity was traced to fermentation occurring in pipe sections (sample valves and thermowell tee) which have since been removed. The problem was diagnosed by the winey odor of 100% NBL cigarettes and confirmed by bacteriological plate count.

The tensile strength of NBL was shown to be affected by the main dryer profile. Higher air velocity or temperatures in Zone 1 of the main dryer induce sheet cracking which reduces tensile strength. Cracking is easily visible and can be avoided. Higher air velocities or temperatures in later zones induce blistering which is more difficult to detect. Even small blisters can reduce the tensile strength from 40 to 20 kg/m. These blisters are readily detected using a microscope with 4x magnification and this technique is being routinely used in the pilot plant.

NBL sheet weight was shown to have no effect on the filling power or smoke analysis of 24% MF blends (all reconstituted tobacco substituted by NBL and production RCB). NBL sheet weights of 110, 130, 150, and 170 gms/m² were evaluated and compared with production RCB at 125 kg/m². This has a significant impact for a capacity increase in the BL Plant.

2. Plans:

Complete evaluation of selected pilot plant NBL products in a Marlboro blend made in the Semiworks – 11/92.

Determine BL Plant conditions for NBL subjective parity with RCB – 3/93.

Install a system in the pilot plant for complete slurry pipework cleaning – 1/93.

Complete the evaluation of recon filler survivability by image analysis – 12/92.

3. Conclusions:

NBL quality is affected by dryer profile.

NBL subjective character is affected by slurry fermentation which can occur by inadequate pipework cleaning at process shutdown.

NBL filling power is not affected by increasing sheet weight.

4. Contributors: Reconstituted Tobacco Development, Chemical Research, Analytical Research, Physical Research, Flavor Technology, Microbiological Research, and BL Plant

5. References: P. Chen, "Quarterly Report – Process Modeling for Drying NBL Cast Sheet," memo to M. Subbiah, November 11, 1992.

Pilot Plant NBL Trials 244–251; 257–259.

Pilot Plant NBL Trials 260–263; 269–272.

Pilot Plant NBL Trials 229, 256.

Pilot Plant NBL Trials 128–130; Series 2–NBL 143, 144; NBL 151, 152; NBL 226–228.

Operations Support (920102), 4th Quarter 1992 from D. Chaddick to R. Ferguson.

B. Strategy: Define the potential formulation reductions of ammonia in the NBL process while maintaining the physical quality improvements of NBL and subjective parity with RCB.

1. Results: The pH of the slurry containing reduced ammonia (10 parts vs. 15 parts/100 parts tobacco) was increased to normal slurry pH by the addition of potassium hydroxide. The sheet quality was maintained but there was a distinct difference in subjective character.

2. Plans:

Evaluate the effect of drying profile on reduced ammonia products.

2021326115

3. Conclusions:

Substitution of ammonia with non-volatile bases is not a viable route to maintain subjective character.

4. Contributors: Reconstituted Tobacco Development, Chemical Research, and Flavor Technology

5. References: Pilot Plant NBL Trial 188-199; 230-233; 236-239

C. Strategy: Develop a recommendation for retrofit of the BL Plant to an NBL process.

1. Results: NBL plant trials will be run in January and February in the BL Plant to achieve subjective parity with RCB and demonstrate improved physical quality. The trials will be run on a single line (Line I) because the dampers on this line can be adjusted while the line is operating. The reverse roll coater roll drives will be modified in December to allow increased roll speeds which have been shown to improve sheet quality in the pilot plant. Several dryer profiles will be run to determine conditions for NBL subjective parity with RCB.

2. Plans:

Select a BL Plant NBL product for POL evaluation - 3/92.

Complete POL evaluation of NBL and engineering development studies - 6/93.

Contribute to the business plan formulation and 650 preparation for BL Plant Modernization by PM Engineering - 12/93.

3. Conclusions:

BL Plant modernization plans are on schedule for completion by 1996.

4. Contributors: Reconstituted Tobacco Development, BL Plant, PM USA Engineering, Physical Research, and Flavor Technology

5. References: Pilot Plant Trial NBL 134; NBL 191

II. Objective: Develop a Cast Leaf process that will provide flexibility in meeting world wide capacity needs for individual reconstituted tobacco types.

A. Strategy: Develop a business plan analysis for Cast Leaf utilization.

1. Results: A combined PME/PM USA meeting was held in October to review global reconstituted sheet needs, OTM utilization and availability, and to determine whether Cast Leaf provides an economic opportunity for the corporation.

Situation analysis showed that PM USA is overutilizing OTM. This generates an economic benefit in the form of green leaf savings so long as OTM availability allows it to be sustained. PME operations in Western Europe (EEC + FTR) are at total utilization on a total pounds basis, but are overutilizing bright stems and scraps, and underutilizing burley stems and dust.

Overall, PM is significantly overutilizing bright stems and scraps. Market prices for these materials are escalating much more rapidly than the prices for green leaf or

offshore strips, and their availability is decreasing. Reduced availability could jeopardize our ability to maintain present incorporation levels of RL, ES/IS and BBO, necessitating their replacement by higher cost green leaf or strips.

The one OTM component readily available at a reasonable price is burley stem. There is also a projected increase in dust availability due to increased recovery of PME factory byproducts, the reclaiming of US stemmery dust, and recovery of tobacco fines at offshore stemming operations. These additional materials are only suited for use in a cast-type sheet product. The additional dust (with burley stems) would provide up to 15 million lbs of cast sheet, which could be produced at the BL Plant if capacity is increased via conversion to NBL.

There was unanimous agreement that this additional cast sheet should be produced and used by the corporation. Given the subjective limitations of RCB, this additional sheet should be Cast Leaf. Cast Leaf would provide the greatest economic benefit if it was used as additional sheet in blends, thereby reducing green leaf and/or offshore strip purchases. Much lower savings result if Cast Leaf is used to replace some mix of RL, ES/IS and BBO; in this instance it merely provides a lower cost feedstock but also reduces our overexposure to the unpredictable availability of scrap/bright stem.

PME presented a utilization scenario wherein their consumption of cast sheet (RCB plus Cast Leaf) would increase by 4 million lbs/year while maintaining total utilization on an overall poundage basis. This would generate no green leaf/strip reduction, but would still provide \$1.5 million/year in feedstock savings, completely utilize their available dust, and reduce their overutilization of bright stems and scraps. PM USA usage of the remaining 11 million lbs of Cast Leaf would equate to an incorporation level of nearly 2% across the board, or higher levels in selected blends. The same use options would apply, i.e., replacement of green leaf or replacement of other processed tobaccos (RL et al) to reduce overutilization of bright stems and scraps.

Review of the EEMA Region situation identified the potential for a 10 million lbs/year Cast Leaf facility to service the Central European Area (Czechoslovakia, Hungary, Poland). A low capital cost facility would be required for economic viability.

2. Plans:

Continue with the Cast Leaf process/product development plan for completion in 1993.

The subjective target of Cast Leaf for PME use will be as an RL substitute.

The business rationale for increased inclusion of cast type sheet will be developed jointly by R&D and PM USA/PME Leaf Departments.

An order of magnitude capital cost estimate for an EEMA Cast Leaf facility will be prepared in 1st Quarter 1993 for review with EEMA management.

3. Conclusions:

Cast Leaf presents a definite economic opportunity for the corporation and development should continue.

2021326117

The mode of Cast Leaf utilization will be affected by sales volumes, product mix, OTM availability and OTM cost.

EEMA will require a low cost Cast Leaf process for economic viability.

4. **Contributors:** Reconstituted Tobacco Development, Development Engineering, BL Plant, PM USA Leaf Department, PME Leaf Department, and PME R&D
5. **References:** Holland, T., "Cast Leaf Program Review – Meeting Minutes – October 6–7, 1992," October 13, 1992.

B. Strategy: Develop a Cast Leaf product with the burn characteristics of RL.

1. **Results:** Laboratory studies showed that pectin release from tobacco could be effected without the use of DAP. The pectin release was effected by various combinations of oxalic acid and ammonia. Hand sheets of satisfactory quality were made with these formulations and pilot plant trials were run to evaluate their processing characteristics. Cast Leaf product was made with oxalic acid substituting DAP while maintaining the ammonia content of the RCB binder solution. Sheet physicals equivalent to NBL were achieved. No improvement in smoke delivery, puff count, and static burn of 100% recon cigarettes was measured despite the lack of DAP. This surprising phenomenon may be explained by the homogenous nature of pectin release products compared with the heterogeneous nature of RL and added binder CL products.

The drying rate of guar gum CL and sheet quality was improved by several means which indicated that slurry stabilization may be the key to the solution of this problem. CL product has the tendency to form a film on the underside of the sheet which separates from the main sheet. This film was determined to be predominately guar gum. Increasing the humectant and sugar content of the formulation and also the addition of one part of methocel or kelcoloid (propylene glycol alginate) reduced this tendency for the guar to separate from the slurry. High humidity drying will also be pursued to improve sheet formation.

2. Plans:

Confirm that pectin release agents other than oxalic acid/ammonia or DAP/ammonia have a slow burn rate and high tar delivery.

Produce pilot plant quantities of steam treated tobacco to evaluate the burn rate and tar delivery characteristics of recon tobacco by this means.

Develop formulations to stabilize CL slurry composition in the dryer to avoid separation of the binder from the sheet and improve drying rate.

3. Conclusions:

Oxalic acid is a viable alternative to DAP for pectin release but does not reduce the tar delivery of cigarettes.

Two families of reconstituted tobaccos exist. One is formed by chemical reaction to give a homogeneous product which has a high tar delivery. The other is formed by

2021326118

physical means which has a more open structure and lower tar deliveries (CL and RL).

4. **Contributors:** Reconstituted Tobacco Development, Flavor Technology, and Chemical Research

5. **References:** Pilot Plant NB 9168, pp 19-21, 31, 35, 36-42; Pilot Plant NB 9041, pp 150-163, 169; Pilot Plant NB 9041, pp 148, 149, 171

Lieberman, P., "Binder Development: Pectin Release," memo to W. Nichols, September 17, 1992.

Lieberman, P., "Binder Development: Pectin Release," memo to G. Keritsis, October 12, 1992.

CLP-224; CLP-234-235; CLP-252-255; CLP-267-268; CLP-273-274

C. Strategy: Support PME Leaf in qualification of LTR for production of RLTC type sheet.

1. **Results:** LTR sheet (LTR 003; RLTC type) has been qualified for use in PME blends. Qualification required a modified routing of the LTR extraction process. Significant production volume at LTR could raise a question as to whether the TC flavor would continue to be sourced in Richmond. PME-R&D has issued a completion report on this project.

2. **Plans:** No further work is planned on this project.

3. **Contributors:** Reconstituted Tobacco Development, PM USA Leaf Department, PME Leaf Department, PME R&D, Flavor Technology, Analytical Research Division, and Park 500

4. **References:** Wagoner, R., "Project SCOTT Final Report," November 2, 1992.

2021326119

2021326120

PROGRAM NUMBER : 920110
 PROGRAM NAME : Tobacco Biochemistry
 PROGRAM COORD. : R. Carchman
 WRITTEN BY : S. Hassam and D. Ayers
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: Efforts to synthesize a model of bound nicotine for testing as a precursor of MS NNK continued on schedule. Conditions under which TSNA could be extracted with supercritical fluids were developed, and results suggestive of supercritical fluid extraction of the MS NNK precursor in base web cigarettes were obtained. Studies of chlorophyll and carotenoid levels in cured fillers of various origins indicated that the curing process smoothed any differences in these tobacco components which may be associated with other filler characterizations, and that further investigations should utilize filler before curing and ageing.

Tobacco plants modified to include antisense constructs of some of the cDNA sequences overly expressed in roots have nicotine levels reduced relative to control. For all plants examined, minor alkaloid levels mirrored the changes in nicotine content. Regenerants which include the 1.2 kb portion of the putative PMT gene in both sense and antisense configuration and appropriate controls have been transferred to the greenhouse. Efforts are now focused on preparing appropriate documentation for patent purposes, completing the sequencing of the complete PMT gene, and, as time permits, incorporation of the PMT gene in an expression vector and measurement of PMT activity in bacteria.

I. Objective: To determine tobacco bound precursor/smoke product relationships for MS NNK by December 1992.

A. Strategy: Devise methods to reduce MS NNK from bound precursors during smoking.

1. Results: The preparation of N'-nitroso-N'-n-butyl-3-picolyamine as a recovery and/or internal standard for TSNA analysis was completed. The preparation, spectroscopic identification and GC-TEA analysis of the compound have been summarized in a memo. Currently TSNA data are reported on an as is basis.

Isolation of a bound form of nicotine from ART burley CEL extracts was investigated using column chromatography (Sephadex G-25 slurry packed in 0.1N NaOH). The separation of (free) nicotine from the rest of the CEL constituents appeared poor. In a another experiment, the S1 fraction of ART burley CEL was extracted with methanol to yield a fraction enriched in a low molecular weight form of bound nicotine. The fraction was further enriched in this bound form of nicotine on a Sephadex G-25 column (eluted with water). Further purification was done on a packed C-18 column followed by RP-HPLC on an ODS-3 column (eluted with methanol/water). A sample has been submitted for NMR analysis.

Experiments to synthesize bound forms of nicotine were continued. Encapsulated nicotine was prepared by polymerizing amylopectin in the presence of nicotine and KOH. The crude encapsulated nicotine was washed with methanol to remove surface nicotine. Solid state NMR analysis of sample prior to washing with methanol showed

2021326121

signals for both encapsulated and surface nicotine. In the washed samples, no nicotine could be observed (the limit of detection for nicotine by solid state NMR is about 1%). The NMR analysis showed that the encapsulated nicotine had been replaced by methanol.

Based on literature procedures, a second experiment was done to synthesize "lignified" nicotine from a reaction of coniferyl alcohol and nicotine in the presence of peroxidase. However, no reaction was observed (possibly due to inactivation of the enzyme by nicotine).

Solid state NMR analyses of acid- and enzyme-treated tobacco marcs are in progress to elucidate the chemical environment of a water-insoluble form of bound nicotine in tobacco.

Investigations to use SCFE to remove TSNA from filler were continued. Previous experiments showed that minor alkaloids and TSNA were not removed efficiently from DBC burley or bright fillers using supercritical (SC) CO₂ alone. No significant reduction of TSNA in DBC Bu was observed using SC 5% MeOH/CO₂ at 75C and pressure < 125 atm. A manual stepwise pressure gradient, from 100 to 300 atm, resulted in a significant reduction in filler TSNA levels (> 90%) with SC 5% MeOH/CO₂. NNK was reduced to below detectable limits. These initial experiments were made difficult due to clogging of the restrictors, apparently by water. Extractions proceeded more smoothly with tobacco that had been desiccated overnight. Reductions in TSNA levels were observed at pressures from 200 to 600 atm. In the case of dried burley tobacco, SCFE at 75C and 300 atm (18 min) led to reductions of 52% in NNN levels, 56% in NAT levels and 61% in NNK levels. Increasing the pressure to 600 atm (22 min) or extending the extraction time at 300 atm (80 min) led to greater reduction in TSNA levels: 70% for NNN and NAT, and 80% for NNK. Similar extractions were done with dried bright tobacco and, at 75C and 300 atm (19 min), NNN levels were reduced by 56%, and NAT and NNK by 61%.

Hoffman *et al.* have reported that SCFE of smokeless tobacco yields more NNK in the extract than conventional methods and have postulated the existence of a bound form of NNK. Previous studies by project 6908 have shown that baseweb has very low levels of preformed TSNA, and that delivery of MS NNK from burley stem base web cigarettes is greater than can be accounted for by preformed TSNA levels. We therefore chose to use SCFE to investigate its effect on MS NNK levels of burley stem baseweb (ASJ). Duplicate SCFE (5% MeOH/CO₂) extractions of the baseweb were done at 600 atm, at two temperatures, 75 and 150C. The MS NNK delivery of the baseweb was reduced by 61% after SCFE at 150C. These observations provide further evidence for an NNK precursor which is reduced by SCFE. The mechanism by which SCFE acts to reduce this precursor in baseweb is unknown.

The peroxidase-release of NNK from a bound source in burley stem baseweb (ASJ) and water-washed DBC burley (APV) was also investigated. Each tobacco substrate was treated with peroxidase in the presence of glucose/glucose oxidase, in acetate buffer. Controls consisted of tobacco, with added glucose, in acetate buffer. After ~ 22 hours, for both tobacco substrates the levels of TSNA in the aqueous phases were

very similar to those of the controls (~80 g/g baseweb; < 30 g/g washed burley). It would therefore appear that no additional NNK from the tobacco substrates was released into the aqueous phase by enzyme treatment. Cigarettes have been prepared from the marcs for MS NNK delivery measurements.

2. **Plans:** Complete documentation of all experiments. No further work is planned for project 6908 in 1993.
3. **Contributors:** S. Haut, R. Izac, R. Forte, R. Kaiser, P. Kurth, S. Hassam, W. Hempfling, ARD.

4. **References:**

Haut, S. Notebook 9200, p.47; p. 75.

Haut, S. Memo to S. B. Hassam, "Preparation of N'-Nitroso-N'-n-butyl-3-picolylamine, a combined recovery/internal standard for TSNA analysis", 11 September 1992.

Izac, R. Notebook 8874, p. 200; Notebook 9215, pp. 1-13.

Forte, R. Notebook 9133, p. 138.

Hassam, S. Notebook 9114, pp. 177; 185-189; 190.

Prokopczyk, B., Wu, M., Cox, J. E. and Hoffmann, D. Carcinogenesis, 13, 863-866, 1992.

II. **Objective:** To design a model cigarette with reduced MS TSNA delivery by the end of 1993.

A. **Strategy:** Reduce MS TSNA by modification of cigarette construction parameters.

1. **Results:** Evaluation of data is in progress.
2. **Plans:** Document data for use as a predictor for MS TSNA delivery of a given cigarette model. No further work is planned for project 6908 in 1993.
3. **Contributors:** R. Izac, J. Tindall.

B. **Strategy:** Reduce MS TSNA by use of tobaccos naturally low in preformed TSNA, minor alkaloids and/or nitrosating agents.

1. **Results:** Analyses of a second series of Japanese and U.S. tobaccos were completed. The fillers were analyzed for TSNA, nicotine and minor alkaloids, nitrates, chlorophyll and carotenoids. As noted previously, filler TSNA levels in Japanese flue-cured tobaccos were significantly lower than U.S. tobaccos and were comparable to those in Izmir tobaccos. Levels of TSNA in Brazil and Zimbabwe flue-cured tobacco were higher than those in Japanese fillers and lower than those in U.S. tobaccos. TSNA levels in Japanese burley tobaccos were lower than in U.S. burley tobaccos. It is probable that differences in agronomic and curing practices (among other factors) may lead to these differences in TSNA levels.

Japanese and U.S. fillers were significantly lower than oriental fillers in chlorophyll levels. Chlorophyll levels ranged from ~190-200 ppm in oriental fillers, 30-100

ppm in flue-cured fillers, and ~10-60 ppm in burley fillers. Differences in chlorophyll levels between Japanese and U.S. fillers were small, on the order of tens of ppm. Chlorophyll level differences between flue-cured and air-cured fillers were also small. Carotenoid levels also did not differ very much between various fillers, ranging from ~120-150 ppm in oriental fillers, ~130-200 ppm in flue-cured fillers, and ~60-190 ppm in burley fillers. This is not surprising as a significant amount (~90%) of the chlorophyll and carotenoids present at harvest are degraded during both flue- and air-curing. In addition, carotenoid levels have been reported to degrade with age in filler. Therefore, pigment differences present during the growing season between tobacco types may not be apparent after curing.

2. **Plans:** Complete documentation of all analyses. No further work is planned for project 6908 in 1993.
3. **Contributors:** W. Hempfling, S. Haut, R. Kaiser, R. Forte, R. Izac, P. Kurth, S. Hassam, PME.
4. **References:** Haut, S. A. Notebook 9200. p.47.
Forte, R. Notebook 9133, p. 131.
Kaiser, R. Notebook 8075, pp. 94-98.

III. Objective: To quantify the effect of cigarette construction parameters and specific additives on the biological activity of CSC by the end of 1992.

- A. Strategy:** Determine the interactive effects of modifications to conventional cigarette construction on the S/M activity of CSC.
1. **Results:** Evaluation of data is in progress.
 2. **Plans:** Complete evaluation and documentation of results. No further work is planned for project 6908 in 1993.
 3. **Contributors:** R. Izac, INBIFO, J. Tindall.
 4. **References:** Izac, R. Notebook 8874, p. 200; Notebook 9215, pp. 1-13.

IV. Objective: Modify tobacco plants so that they produce reduced levels of alkaloids as compared to the cultivars currently in use by December, 1995 (PMT-based modification, December, 1993 and an MPO-based modification, December, 1995).

- A. Strategy:** Influence tobacco biochemistry by expressing the antisense of the cDNA sequences that are overly expressed in tobacco root to reduce the level of alkaloids in tobacco plants.
1. **Results:** Additional differential hybridization regenerants have been assayed for pre- and post-topping alkaloid content to identify those with significantly reduced levels. The results for the 4 regenerants with the 50A construct were very similar to the controls. In contrast, the 4 regenerants containing the 19A construct accumulated

18 - 82 % less nicotine. Five regenerants containing the 10.6 antisense fragment displayed on average almost twice the nicotine of the controls while the full length insert was found to accumulate 83% less nicotine than the controls. In all cases, changes in minor alkaloid levels mirrored the observed changes in nicotine content. Alkaloid analyses on additional differential hybridization regenerants is pending.

In order to assess the heritability of a trait altered genetically, seed from a differential hybridization regenerant (17.2a1-4) were selected. A total of 39 transformed plantlets, 22 Burley 21 controls and 5 pBI121 controls are presently being grown in the greenhouse. These plants will be assayed for alkaloid content pre- and post-topping.

2. **Plans:** Continue alkaloid analyses on transformed plants.

3. **Contributors:** M. Shulleeta, B. Vaughan, B. Forte, Greenhouse personnel

4. **References:**

Shulleeta, M. Notebook 9132.

Forte, R. Notebook No. 9133, pp. 131, 138.

B. Strategy: Express the antisense of the DNA sequences for PMT in tobacco plants.

1. **Results:** The feasibility of altering alkaloid levels in transformed plants by introducing the 1.2 kb PMT construct in either sense or antisense orientation is currently being studied. To date 20 antisense regenerants (1.2A) and 15 sense regenerants (1.2S) along with 15 pBI121 controls have been transferred to the greenhouse for growth, alkaloid sampling and seed set.

2. **Plans:** Transfer an additional 20 plants of each type described above to the greenhouse. Conduct alkaloid analysis and collect seed from these plants.

3. **Contributors:** M. Shulleeta, B. Vaughan, greenhouse personnel.

4. **References:** Shulleeta, M. Notebook No. 9132.

C. Strategy: Continue to use PCR technology and the DNA sequence data to isolate the intact putative PMT gene. Complete the sequencing of the intact putative PMT gene. Clone the intact putative PMT gene into an expression vector and test for expression of PMT. Further characterize antibody produced against the synthetic 29 amino acid polypeptide and the 60 kD band of the putative PMT protein.

1. **Results:** The full-length (~1.5 kb) putative PMT gene was cloned into pBluescript II (SK+) (for sequencing) and a bacterial expression vector, pTrc 99A. Several preliminary protein expression experiments were carried out using the bacterial expression vector with the cloned PMT gene (pTrc-PMT14-3). Based on protein gels and PMT activity assays, it appears that there was no definitive PMT expression in these experiments. Different expression vectors will be tried if time permits (2).

The full-length PMT gene is being sequenced. Several additional primers were obtained to facilitate sequencing of the internal portion of the gene. Comparison of

2021326125

partial sequence data obtained from several separate isolates suggest that PMT may be a member of a multigene family (2).

Information regarding the PMT patent (1412) was supplied to the PM lawyers. Additional information regarding the potential patent of the root overly expressed genes was also provided.

Due to other priorities work on the antibody generated against the synthetic 29 amino acid polypeptide and the 60 kD band of the putative PMT protein was suspended (1).

2. **Plans:** Complete sequencing of the PMT gene. If time permits, test the PMT gene in additional expression vectors.
3. **Contributors:** M. Krauss, T. Michalik, S. Wahab
4. **References:** Krauss, M. Notebook No. 9204.
Wahab, S. Notebook No. 9201.

2021326126

**ENVIRONMENTAL
SUPPORT**

2021326127

PROGRAM NUMBER : 920111
 PROGRAM NAME : Environmental Support
 PROGRAM COORD. : C. R. Hayward
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: Previous development work showed that water scrubbing was effective in removing the VOC's and nicotine from the BL Plant stack gases. Evaluation of biotreatment of the scrubber water has been shown to be quite effective in removing the nicotine, PG, and ammonia from this stream. In 1993, a new type of scrubber will be installed at the BL Plant to develop final design data for Engineering.

The Burley stem washing has been discontinued, so no further effort is planned in this area.

Tests in 55 gallon drums at Park 500 showed that it was feasible to develop a bioremediation process for removing tobacco alkaloids from the Park 500 combined sludges with adequate aeration.

Analytical support of the environmental objectives continue. An on-line GC compared favorably with the EPA method for determining VOC emissions from the aftercut cylinders. TCLP analysis of solid wastes indicate that the dried Park 500 sludge and the spent catalyst for phosphine oxidation can be discarded as a non-hazardous waste.

A project is starting to support Engineering's development of a catalyst to oxidize phosphine.

I. Objective: Develop technology to reduce the levels of various components (nicotine, ammonia, and VOC's) in air emissions at the BL Plant to support Environmental Engineering in maintaining compliance at the site and preparing for future permitting requirements.

A. Strategy: Evaluate adsorption technology for either direct treatment of air streams or for treating blowdown from a scrubber.

1. Results: Regeneration of a XAD-4 resin loaded with 17% nicotine using steam was investigated. The use of low pressure house steam (212-250°F) revealed only a 26% removal efficiency in 4 bed volumes of steam condensate. The use of steam is less efficient than 95% ethanol which removes greater than 90% of the nicotine from the resin in 4 bed volumes.

As reported last quarter, adsorption technology has been eliminated as an applicable technology for the BL Plant emissions. While resins are available that can remove the nicotine from scrubber blowdown, ammonia and PG removal are not feasible.

2. Plans: No further tests are planned.

3. Contributors: Reconstituted Tobacco, Chemical Research

B. Strategy: Evaluate scrubbing technology for treating the air discharges.

1. Results: Design information for a scrubbing system for the BL Plant was transmitted to Engineering, and they have prepared preliminary cost estimates for scrubbing as

2021326128

well as for incineration and a dispersion scenario based on tall stacks. Because of the complexity of the permitting situation, as well as the implementation of the NBL process, management has decided to submit the permit application and appropriation request in January 1994.

The installation of a pilot scrubbing unit (manufactured by Sly Manufacturing) has been postponed until next March. This unit, which will develop the final design data for the scrubber, was originally to be installed in December. However, with the permit submission timing, this evaluation was delayed.

Work during the past quarter has concentrated on the activated sludge treatment process for the wastewater from a scrubbing system. The pilot scrubber at the BL Plant has been operated to provide a feed for a 100-gallon pilot sequential batch reactor (SBR) for this evaluation.

Results have been very encouraging. The pilot SBR has been operating for over three months with effluent showing no detectable nicotine, less than 1 ppm of ammonia nitrogen, and chemical oxygen demand (COD) in the 100-150 range. The low nicotine and ammonia results have been consistent despite changes in the feed concentrations, disruptions in the feed lasting several days, power outages affecting the pilot system, and occasional pH excursions while parameters were being fine-tuned. No toxic problems have been encountered, so the system is quite robust with respect to upsets in the operation.

The system was started in August treating the pilot scrubber wastewater. The pilot scrubber is treating the south main dryer stack at the plant which contains a higher concentration of PG and nicotine than the combined five stacks that would be treated in a full-scale operation. As a result, this wastewater contains higher levels of PG and nicotine, and thus COD. The ratio of COD to ammonia in this wastewater was about 40:1.

In late September, after over six weeks of successfully treating this wastewater, the feed was modified by dilution and spiking with ammonia to simulate the wastewater from a scrubber treating the combined five stacks. This feed has the same ammonia concentration but only about half the PG, so the COD to ammonia ratio is about 22:1.

The system adjusted to the new feed with no problems as the effluent concentrations remained low. The more dilute feed has required the addition of alkalinity to maintain the proper pH range in the reactor and has improved the settling properties of the biomass. Both of these results were expected, and in general, the system has performed very well and with no unforeseen problems.

The BL Plant implemented a reduced PG formulation in November that will further reduce the PG concentration in scrubber wastewater. Tests are now in progress to quantify this reduction to determine the expected wastewater concentrations from a full-scale scrubber.

- Plans:** Once these new concentrations are identified, the feed to the pilot SBR will be modified to reflect the changes and the system's performance again monitored. During the next quarter, we will also be investigating other issues in the wastewater treatment process: the oxygen transfer efficiency in the system, quantifying any

2021326129

volatiles evolved from the aeration basin, and optimum startup procedures. The wastewater treatment process development will be completed in early second quarter 1993.

The pilot Sly scrubber will be received for installation and testing in March.

3. **Conclusions:** Scrubbing and biotreatment of the wastewater continues to be a viable and attractive alternative for treating the BL Plant air discharges. No unexpected problems have been found with the wastewater treatment process.

4. **Contributors:** Reconstituted Tobacco Development

II. **Objective:** Develop alternatives for treatment of Burley stem washing effluent.

A. **Strategy:** Evaluate elimination of the Burley stem washing process for RCB.

1. **Results:** Flavor Technology has completed the subjective evaluation, and the Burley stem washing extract is no longer being sewered.

2. **Plans:** No further work is planned.

3. **Contributors:** Product Evaluation, Flavor Development, Reconstituted Tobacco

III. **Objective:** Develop a plan to reduce or eliminate potential environmental and disposal problems associated with the potassium nitrate co-product from Park 500.

A. **Results:** The feasibility of using adsorption technology to improve the quality of the co-product (potassium nitrate) produced at Park 500 has been proven in the laboratory. The loading capacity using the XAD-4 resin in this process will be determined.

Recrystallization of the potassium nitrate has been shown to improve the quality of the co-product.

B. **Plans:** Complete the loading capacity study using the XAD-4 resin.

C. **Contributors:** Chemical Research Division

IV. **Objective:** Support the R&D and Engineering Five Year Plans addressing air, water, and solid waste issues.

A. **Strategy:** Provide analytical expertise related to sampling protocols and analytical schemes for qualitative and quantitative descriptions of air, water, and solid waste effluents.

1. **Results:**

Effluents – Nicotine was determined by HPLC for the samples taken during the September–October Park 500 effluent monitoring test. The analytical data included appropriate information necessary to calculate the quality assurance aspects of the series and were sent to personnel at ERM for inclusion in the final report.

2021326130

A portable gas chromatograph was evaluated for on-line speciation of low-boiling components in stack effluents. Quantitative values for ethanol emissions in the aftercut cylinder exhaust were calculated at the 900 ppm range. These values compared favorably with the 1000 ppm range reported by the EPA Method 25A (total VOC by direct flame detection).

Sludge - Nicotine, ammonia, and VOC's were quantitated in the condensate derived from drying samples of 'Centrifuge Sludge' and 'Belt Press Sludge'. The VOC values were derived by purge and trap techniques and determined by quantitatively comparing the sum of the areas of all gas chromatograph peaks to the response for pentane in water. Nicotine was determined by HPLC and ammonia by gas sensing electrode.

2. **Plans:** Samples from the November effluent tests at Louisville have been received and are being prepared for analysis.

Quality Assurance Check samples will be prepared at R&D for continuing confirmation of data produced by the consulting labs, especially as applied to on-site analyses by their mobile lab facilities.

3. **Contributors:** Analytical Research Division

- B. **Strategy:** Support Environmental Engineering's development of a catalytic carbon process to oxidize phosphine. Evaluate alternate processes that have an economic advantage over the catalytic carbon process.

1. **Results:** The initial laboratory equipment and necessary safety requirements have been compiled to evaluate alternate methods to remove phosphine from the air after fumigation of tobacco warehouses.

2. **Plans:** Locate commercial suppliers of supported oxidation catalysts. Determine the best method to detect and measure phosphine for both laboratory and field studies. Construct more highly sophisticated equipment to allow more refined studies to be conducted. Investigate the synthesis of new supported oxidation catalysts. Investigate the feasibility of using solution oxidation chemistry.

3. **Contributors:** Chemical Research Division, Analytical Research Division

- C. **Strategy:** Follow the TCLP protocol, collect, analyze, and evaluate the data generated within PM and at the various contract laboratories.

TCLP Analysis of Silver Impregnated Carbon

Results: A sample of virgin silver impregnated carbon used in the July warehouse fumigation scrubbing study was extracted by the TCLP protocol and examined for silver content. The silver content of the extract was below the TCLP limit, and considering that no other regulated component should be present in the virgin material, the material would be classified as a non-hazardous waste.

Plans: No additional studies are planned for this material.

Contributors: Chemical Research Division, Analytical Research Division

2021326131

TCLP Analysis of Dried Biosolids (Sludge)

Results: The two samples of dried biosolids prepared by Environmental Engineering were analyzed by the TCLP protocol at two contract laboratories. The data has been received and reviewed. The content of the components of interest of the extracts were all below the TCLP limit. Based on these results, each sample would be considered non-toxic.

Plans: No additional toxicity characteristic studies are planned for this material.

Contributors: Chemical Research Division, Analytical Research Division

Contract Laboratory Certification for TCLP Analyses

Results: Two contract laboratories, IEA located in Research Triangle Park, NC and Environmental Laboratories, Inc. located in Richmond were evaluated as part of the TCLP analysis of the dried biosolid (sludge) samples. The quality of the laboratories were determined by 1) comparing metals analyses between the contract labs and the results obtained by ARD, 2) comparing other analyses between contract labs, 3) evaluating spike recovery data, 4) evaluating how well they worked with PM on this project, and 5) evaluating their timeliness and quality of their reports. Based on the above criteria, Environmental Laboratories, Inc. was the preferred laboratory.

Plans: Monitoring of the contract laboratories will occur on a continuing basis.

Contributors: Chemical Research Division, Analytical Research Division

V. Objective: To evaluate the use of microorganisms to reduce the levels of selected compounds in different processes.

A. Strategy: Determine the bioremediation conditions required to remove tobacco alkaloids (TA) from the centrifuge sludge (CS) coming from the Park 500 process.

1. Results: CS, press cake (PC), and KNO_3 were mixed at the ratio of 1:2:0.05 in two 55 gallon drums and CS and PC were mixed at the ratio of 1:2 in one 55 gallon drum. In this one drum study, run in the Park 500 Waste Treatment Sludge Dewatering building, TA reduction occurred, after 13 days, in all 3 drums at the following percentages:

Sludge Mix	Top 1 Inch	Middle 15 Inches	Bottom 30 Inches
CS:PC: KNO_3			
(Stirred)	100.0	78.7	76.8
(Unstirred)	96.4	74.1	70.3
CS:PC			
(Unstirred)	93.3	60.9	56.9

2. Plans: None at this time.

2021326132

3. **Conclusions:** TA in the top layer of a CS-PC-KNO₃ sludge mixture can be removed to non-detectable levels in 13 days with proper mixing (aeration).
4. **Contributors:** Biochemical Research Division

2021326133

NEW
PRIMARY PROCESS

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PROGRAM NUMBER : 920112
 PROGRAM NAME : New Primary Process
 PROGRAM COORD. : S. E. Clark
 PREPARED BY : C. A. Wood
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: Expenditures against the approved 650 for Semiworks modifications began. A filler addback blending line was constructed and started. The continuous microwave unit was integrated with an SRB force feed and the Millicutter. A Panda superheated steaming tunnel was installed in D Pilot Plant prior to the Frigoscandia unit. Design work was initiated for shutdown installation of an in-line blending unit and a tub blending system. A weigh belt was installed exit the DCC for casing application control.

A Unit Operations focus has continued this past quarter in the areas of microwave heating prior to cutting, filling value enhancement, filler survivability, and flavor system definition for individual components.

The emphasis, begun last quarter, to identify and evaluate the potential for incorporating individual component processing concepts into existing facilities has continued. The weight savings potential of up to 35 mg for price value products was repeatedly demonstrated with the steaming tunnel treatment prior to total blend cut filler drying. Subjective differences continue to be evident even at less severe tunnel operating conditions and will likely delay the initiative to seek funding for converting two existing facilities to this processing concept by the end of 1993.

Synergies with the NET/SCI program in the areas of partial expansion of filler blends and alternate treatment following impregnation were explored. Additional work in this area is warranted based on preliminary results.

The scope of the New Primary Process program has been enlarged and the program has been retitled. The Primary Technology Program will incorporate the NPP concepts with other tobacco utilization and processing strategies that increase cigarette yield from tobacco purchased, increase processing flexibility, and reduce the cost of cigarettes produced.

I. Objective: Develop a process for preparing blend components individually or in selected combinations which manages the interaction objectives of maximizing the cigarettes produced per pound of tobacco utilized and achieving subjective acceptability of the blend.

A. Strategy: Utilizing various blending techniques, demonstrate that individually processed components can be blended to a degree of uniformity that is at least comparable to current total blend process methods.

1. Results: Previous studies utilizing natural chemical markers found no blend differences between cigarettes produced via individual component processing (ICP) and the conventional strip blending process. Picking analysis of the cigarettes found no indication of differences in blend uniformity.

The existing three wheel carding unit was incorporated into the modified Semiworks addback area to declump 100% cut RL prior to blending with the remaining blend components.

Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Fluorescence (XRF) analyses of six grindings of the Bokelman sample set are nearly completed. These data will be used to develop a preliminary calibration for blend composition of single cigarette rods. Early evaluations using the PLS statistical method indicate that all components can be quantified to some level of precision. CAD is providing assistance in defining the wavelength regions which are most unique in characterizing the various components. These efforts should significantly reduce data processing time for calibration. The standard deviation will be used to measure the blend uniformity.

2. **Plans:** An increased capacity, six wheel blending unit has been designed and will be installed in Semiworks to provide in-line blending of the combined components. Control testing will begin in December.

Utilizing FTIR and XRF techniques, estimate the level of measurement precision for each of the eight blend components based on the Bokelman sample set, to establish a calibration using the BRICA blend components. Continue to refine the procedure to achieve the highest possible precision.

Additional data will be collected to investigate the blending efficiency of the new Semiworks cut component blending line utilizing FTIR and XRF techniques in January.

3. **Conclusions:** To date, measures of blend uniformity by picking and chemical markers have provided no indication of blending differences between individual component processing and conventional strip processing. Utilizing FTIR and XRF methods to investigate the blending efficiency of the new Semiworks cut component blending line on individual cigarettes will provide improved techniques for blend consistency determinations.

4. **Contributors:** B. Kanipe, D. Lisbon, R. Mullins

- B. **Strategy:** Identify by component, specific operational parameters which provide improved strand length and cigarette firmness. Set priorities for unit operation activities based on their potential impact on cut component physical quality performance.

1. **Results:** Tests designed to determine the operating parameter settings for the present Semiworks configuration which yield maximum filling value for each component have been completed for BRICA Bright, Burley, and Oriental tobaccos. The test matrix, designed by PED and consisting of 15 tests per component, examined the effect of Hauni tunnel residence time (15 to 60 sec), bed depth (0.5 to 1.5 inches) and steam/tobacco ratio (0.3, 0.4, and 0.5) on filling value. All other cutting-steaming-drying parameters were set as prescribed by the screening test results for best filling value. The exit silo CV's showed that processing through the tunnel enhances filling value, but the effect is not significantly affected by changes in residence time, bed depth, or the steam/tobacco ratios tested.

Cigarettes for subjective evaluation have been made with 100% Oriental tobacco taken from a control not processed through the Hauni tunnel and from five of the tests representing the extreme conditions of processing.

2021326136

During the week of November 16, 1992, cigarettes were made with various levels of Expanded Tobacco which have been processed at several tower temperatures. Three blends will target a weight reduction of 60 mg/cigt, and three blends will target a weight reduction of 100 mg/cigt. The -60 mg blends will utilize 22CU (24 sec) cigarette paper (Control uses 33CU/16 sec), and the blends targeting -100 mg weight reduction will use a cigarette paper supplied by Paper Technology which was obtained from Ecusta and has a porosity of 26CU and 0.55% MAP. This paper is used commercially on Doral Lights.

C. Strategy: Conduct laboratory tests directed toward identifying and obtaining a better understanding of the mechanisms which influence filling power.

1. Results: The effects of the moisture content and temperature on the stress-strain properties of Bright lamina indicated improved plasticity at elevated temperatures above 150°F, which would be favorable for low OV, high temperature processing.

The dynastat sample chamber was completely redesigned. The new chamber provides a more uniform condition throughout the chamber and is expected to better control the temperature and moisture content of a test sample.

It is not possible to determine the mechanical properties of individual shreds of RL and RCB because of significant sample-to-sample variation in shred integrity.

2. Plans: Stress-strain tests are progressing well on Burley, Oriental, RL, RLTC, and RCB at 65% Rh and the same temperatures at which the Bright was measured. This effort will be completed by December 1, 1992. The time dependent CV measurements will be made after the completion of stress-strain tests. The work will be done in parallel with the creep recovery tests to determine if the impact of shred stiffening and curl can be quantified. These tests are expected to be completed by the end of January. The creep recovery tests to determine the stress relaxation times as a function of Rh and temperature will then begin and are expected to be completed by the end of January 1993.

3. Contributors: A. Basak, S. Ganeriwala

4. References: A. K. Basak, S. N. Ganeriwala to J. N. Crump, Memo, "Mechanical Properties of Tobacco Leaf Lamina," October 12, 1992.

D. Strategy: Evaluate the feasibility of applying microwave heating to tobacco strip prior to cutting at reduced moisture levels.

1. Results: The microwave heating unit at the Semiworks was modified to continuously feed heated tobacco strip to the Legg Millicutter at a rate of approximately 300 pounds per hour. This modification was completed and successfully started up in October. A test was conducted to compare the Hauni steam tunnel versus the Panda superheated steam tunnel as processing scenarios after the microwave heating and cutting steps to open the cut rag. 1500 lbs of Bright strip was heated to approximately 160°F at the microwave and cut at 140°F and 17% OV at the Millicutter. Part of the cut rag was processed through the Hauni steam tunnel and dried to 13% OV via the

2021326137

Hauni KKK dryer. The other portion of the cut rag was processed through the Panda superheated steam tunnel and dried to 13% OV via the Frigoscandia unit at D Pilot. Samples were taken to characterize moisture profiles through the process as well as filling power characteristics of the finished product. Samples were also taken to monitor various chemical markers to establish changes resulting from the process. Results from the test are expected within the next week.

2. **Plans:** Complete the analysis for the Bright test described above as well as characterization of the other tobacco components to establish the potential for utilizing this technology in low moisture processing within the New Primary Process.

Based on the results from this test, similar tests are planned to process the other tobacco components using cased strip from the DCC opening and casing process in order to fully characterize the moisture profiles and filling power characteristics through the entire processing path.

3. **Conclusions:** Tobacco strip was successfully heated and cut at a reduced moisture level using the microwave heating and cutting process. This gives us the tools to establish the potential for managing the moisture profile, establish the filling power characteristics and establish changes in the chemical makeup of the tobacco and reduce capital requirements for a New Primary Process.

- E. **Strategy:** Develop a database to record processing parameters and to provide access to analytical data for New Primary samples.

1. **Results:** The database and the data entry forms have been created.

2. **Plans:** NPP staff will be trained to use the database system in December.

3. **Contributors:** A. Lewis

- II. **Objective:** Develop casing and flavoring systems that complement the process developed in this program and achieve the subjective acceptability necessary to produce existing and additional value added products.

- A. **Strategy:** Develop flavor systems to achieve subjective parity between individual cut component (ICP) processing and total blend processing.

Formulate flavor systems to achieve lower costs and reduced thermal treatment through consolidation of Unit Operations.

Consolidate flavor systems to reduce cut component storage needs.

1. **Results:** Models were produced with casing reformulation/reallocation to remove the sensory differences noted with individual cut component processing. A confirmation run of the most promising model is pending subjective evaluation.

Modifications are complete on the DCC flavor system to allow casing application to non-steady state product flows. Combining the conditioning and casing in the DCC in combination with other NPP process changes aimed at reducing moisture gains will eliminate the need for the traditional Bright Casing and Burley Spray/Burley Drying operations.

2021326138

Two series of reduced humectant models (ICP and Total Blend processing) were made to measure the effect on filler physicals and subjectives. The first series (ICP) indicated lower survivability at the lower humectant levels with a trend toward higher equilibrated firmness. Subjective smoking by the NPP Panel of the first series indicated a significant sensory sequence effect.

2. **Plans:** Demonstrate subjective acceptability for Price Value Products between ICP and conventional processing through complimentary flavor and casing system in large scale Semiworks by March 1993.

Qualify the DCC as an alternative casing system for Bright and Oriental by February 1993.

Simulate targeted NPP casing scenarios during first quarter 1993. DCC application and split Burley Spray (second application to cut rag) will be used to eliminate the Bright Casing cylinder, Burley Spray cylinder, P&S dryer, and Top Casing cylinder.

Issue recommendations for reducing humectant levels in casings by the end of the first quarter of 1993.

3. **Contributors:** B. Bell, D. Ennis, H. Spielberg, D. Rockwell, R. Pitts

- B. **Strategy:** To provide expert and consumer testing of cigarette models resulting from the development of the New Primary Process (NPP) at various stages in its development. To establish the sensory basis for qualifying the NPP as a viable alternative to the current primary process and to develop an understanding of the effect of unit operation variables on cigarette sensory parameters.

1. **Results:** Plans from the third quarter were implemented concerning the development of a method for difference detection based on "same-different" judgments using the single puff smoking method. BRICA cigarettes involving different steam/tobacco ratio treatments were tested and (a measure of sensory difference) was estimated. Small to large systematic differences were observed between a control and 0.2, 0.3 and 0.4 steam/tobacco ratio models. Unusual sequence effects were observed in a BRICA humectant level study that required the development of an extension to the model for "same-different" judgments. Sequence effects (probably due to a form of adaptation) were parameterized by allowing a prior cigarette to alter the variance of a succeeding cigarette. It was also noted that control/control and test/test pairs did not always produce the same probability of reporting a "same" response. Two new parameters, r (the ratio of the detection criteria used to decide if a pair is to be called "different") and λ , a variance multiplier, were found sufficient to explain these results. Under the assumption that two cigarettes are identical, $\lambda = 0$, $\lambda = 1$ and $r = 1$. In the humectant level study, λ was not significantly different from 0, but λ was significantly different from 1. This kind of result suggests that it is possible to perceive differences between cigarettes that arise as a result of direct comparisons but that may not be evident if cigarettes are evaluated in isolation (as consumers usually evaluate them). However, if *any* of these parameters are significantly different from their expected values under the null hypothesis (those given above), the cigarettes must not be identical. Bright cigarettes from tobacco subjected to different tunnel treatments

2021326139

(steam/tobacco, bed depth and residence time) were tested using "same-different" judgments and the results were analyzed to estimate σ , and r . These results will be reviewed before the end of November with the NPP Team. New experiments involving a 35 mg tobacco weight reduction have been initiated.

2. **Plans:** Work on refinement of the single puff smoking method will continue. One issue requiring resolution is the need for interpuff rinsing. This issue is currently being addressed using the weight reduction cigarettes. A prototype computer-based data entry and analysis capability will be developed before the end of 1992. Further work on the other single components subjected to tunnel treatment will be conducted. Assuming continued staff availability, a new NPP panel will be created.

3. **Contributors:** D. Ennis, B. Joyner, C. Hayes, G. McLaughlin

- C. **Strategy:** To provide analytical data that will support the optimization of Physicals, Control Taste, and Enhance Flavor Reallocation in the development of New Primary Processing Technologies.

1. **Results:** Burley Strip before and after Burley Spray for comparison with NIR Sugar Monitor were analyzed for OV and IC-Sugars.

Frigoscandia Drying Trials samples were analyzed for Alkaloids, Reducing Sugars, and OV.

Cut and Dry Optimization test using BRICA Bright, BRICA Burley, and BRICA Oriental samples were analyzed for Alkaloids, Reducing Sugars, and OV.

Cased BRICA Bright Panda Test samples were analyzed for Alkaloids, Nicotine, Reducing Sugars, Glucose, Fructose, Sucrose, and OV.

Microwave heating and cutting of cased BRICA Bright processed through the Hauni tunnel and the Panda-Frigoscandia drying samples were analyzed for Alkaloids, Nicotine, Reducing Sugars, Glucose, Fructose, Sucrose, Karl Fischer Water, and OV.

Relative Humidity Chamber for Expanded Tobacco samples were analyzed for Alkaloids, Reducing Sugars, and OV.

Static Test using Uncased Components samples were analyzed for Alkaloids, Reducing Sugars, and OV.

An X-Ray Fluorescence (XRF) Method is in development to aid in the determination of blend composition. Samples are being analyzed to further elucidate the method and maximize operating parameters.

2. **Plans:** To continue to establish Sampling Protocols which will better maintain sample integrity prior to delivery to the Chemical Analysis Section for analysis. To continue to support New Primary Processing Concepts and Design associated with Optimization of Physicals, Controlling Taste, and Flavor Reallocation.

3. **Contributors:** N. Adams, L. Branch, C. Callicutt, A. Ganzert, R. Jones, S. Langley, T. Larus, M. Mangrum, R. McDaniel, B. Ryan, D. Self, K. Torrence

- III. **Objective:** Develop processing scenarios that maximize productivity, efficiency, and flexibility.

2021326140

A. **Strategy:** Develop computer models of New Primary and Existing Primary Processes which can be used to optimize the logistics of our Primary/Manufacturing operations.

1. **Results:** A process simulation model of the current Cabarrus facility is being validated against actual Cabarrus operation to verify the accuracy of the model. The collection of plant statistics and operating rules is underway.
2. **Plans:** Modify the model to incorporate existing Cabarrus plant information and operating rules and verify model accuracy against actual Cabarrus operation. In cooperation with Cabarrus personnel, explore alternative approaches to their planned capacity expansion, and test new scheduling approaches in Primary and Cigarette Manufacturing.
3. **Conclusions:** Factory simulation models promise to be an important aid in planned capacity expansion and in optimizing the logistics of our Primary and Cigarette Manufacturing operations.
4. **Contributors:** L. Haws

IV. **Objective:** Identify and recommend technologies which evolve out of the New Primary Process development for Total Blend processing that could be implemented within existing facilities to achieve improved tobacco quality, yield, and result in reduced cost per thousand cigarettes produced.

A. **Strategy:** With initial emphasis on price value products, develop new processes and product specifications to improve the tobacco weight versus firmness relationships with acceptable subjective response.

1. **Results:** BRICA total blend, steam treated, reduced weight cigarette models were produced incorporating various design changes to allow the cigarettes to match target puff counts, deliveries, and subjectives. Evaluation of the models indicated that puff counts and deliveries can be matched acceptably. Subjective differences attributable to both the tunnel treatment and control cigarettes at reduced weight were detected and have not been resolved.

Potential implementation scenarios and schedules for introducing the technology into Manufacturing were developed in cooperation with Engineering, Production Planning, and Manufacturing. The tunnel sizes could not be completely specified because the Semiworks trials were conducted with much longer residence times than are practical for factory operations. The Semiworks tunnel was modified to allow operation at residence times down to 7 seconds and a study initiated to determine the impact of reduced residence times on cigarette weight savings. Production of all filler and cigarettes for this study has been completed along with testing of approximately one-third of the samples.

Cambridge Full Flavor 100's, Cambridge Lights 100's, and Cambridge Ultralights 100's were also produced at a control and -35 mg weight target. All brands utilized 27CU (19 sec) cigarette paper for both the Control and Hauni treated product. FTC Tar test cigarette values were comparable to the Control, but tended to be on the low side of target.

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During the week of November 16, 1992, cigarettes were made with various levels of Expanded Tobacco which have been processed at several tower temperatures. Three blends will target a weight reduction of 60 mg/cigt, and three blends will target a weight reduction of 100 mg/cigt. The -60 mg blends will utilize 22CU (24 sec) cigarette paper (Control uses 33CU/16 sec), and the blends targeting -100 mg weight reduction will use a cigarette paper supplied by Paper Technology which was obtained from Ecusta and has a porosity of 26CU and 0.55% MAP. This paper is used commercially on Doral Lights.

2. **Plans:** Perform additional filler analytical and smoke chemistry analysis which may identify chemical differences resulting from steam tunnel treatment. Additional modifications and/or reallocations of flavor systems will be evaluated that may favorably impact subjectives.

The weight reduction limit will be established beyond which subjective changes occur in a control cigarette. The impact of shred width on subjective response in a reduced weight cigarette will also be evaluated.

Specifications for production rated steam tunnels will be formulated. The implementation scenarios for the factory with the input of Production Planning, Manufacturing, and Engineering will be refined to reflect initiation of installations in December 1993.

3. **Conclusions:** The implementation of Steam Tunnels prior to cut filler dryers offers significant weight savings potential. Due to subjective differences which were detected on Cambridge reduced weight control models as well as tunnel produced fillers, joint efforts between Product, Process Development, and Flavor Technology will concentrate on establishing subjective targets and producing samples of subjectively modified cigarette models to achieve acceptability for the various BRICA blend cigarette constructions utilizing the steam tunnel process.

4. **Contributors:** J. Crump, D. Lisbon, R. Mullins, D. Rockwell

- B. **Strategy:** Utilize the current continuous microwave process in Semiworks Primary to heat filler prior to the maker hopper for improved filling power and shred survivability. Based on these results, determine the potential for Radio Frequency heating of filler at the maker and develop prototype commercial designs.

1. **Results:** A control and two samples were tested. Both samples indicated weight reduction potentials of 10 mg and 15 mg, respectively. Exit gamiture OV's were comparable in all cases. Anethole and ethanol were tracked throughout the process. No significant loss in Anethole was observed. Ethanol was not detectable in cigarettes in any of the three cases.
2. **Plans:** In collaboration with Engineering, develop costs for implementation of an RF heater on both the Mark-9 and Protos makers. Identify implementation strategies for current cigarette making operations.

2021326142

3. **Conclusions:** Elevated moistures and temperature to the maker increases tobacco pliability. In this case, a minimum weight reduction of 10 mg was associated with the test conditions.
4. **Contributors:** T. Howell, D. Lisbon, C. Wood

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2021326144

**SENSORY
TECHNOLOGY**

PROGRAM NUMBER : 920113
 PROGRAM NAME : Sensory Technology
 PROGRAM COORD. : R. Carchman
 WRITTEN BY : C. Hayes, W. Reininghaus, J. Garman, J. Lephardt
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: The sensory evoked potential lab is being reestablished at INBIFO. The development of analytical procedures to measure the acid/base character of filler and smoke continue as scheduled, but filler basification studies have been delayed due to other priorities.

I. Objective: To develop the technology to produce low alkaloid and low tar/low alkaloid cigarettes that have sensory characteristics superior to currently available products.

A. Strategy: Develop a fundamental understanding of how nicotine affects sensory systems.

1. Results: Concentration response curves for both (*S*)- and (*R*)-nicotine have been determined for the frog nasal epithelium by the Kobal group in Erlangen. A concentration response curve for low concentrations (5 – 150 g/l) of (*S*)-nicotine is in the process of being determined by INBIFO for the nasal epithelium of the frog.

2. Plans: Compare the sensory characteristics of low concentrations of (*R*)-nicotine to the same concentrations of (*S*)-nicotine in the frog model at INBIFO. Conduct collaborative studies with the Kobal group on cognitive evoked potentials to odorants. Establish a patch clamp recording facility in Erlangen in order to study selected compounds with neuronal cell cultures.

3. Contributors: INBIFO, Erlangen.

B. Strategy: Develop an analytical approach to the measurement of the acid/base character of filler and smoke.

1. Results: Due to the high priority placed on the De-Nic menthol program, filler basification studies have been delayed. However, an investigation has been reinitiated to determine whether a $\text{Ca}(\text{OH})_2$ /sucrose casing formulation will yield a stable filler of the desired pH and be suitable for scaled up applications (1).

Comparative testing has shown that gas phase smoke is more acidic than whole smoke. The addition of a surfactant (Brij 35) to the pH 7 buffer solution resulted in no significant difference in the measured acid/base character of the smoke or analytical performance. The addition of KCl to the pH 7 buffer solution is currently being evaluated.

An alternative "smoke pH" measurement, employing direct impaction onto a pH electrode, was investigated. The approach was abandoned, however, when between puff rinsing of condensed material from the electrode was insufficient to reestablish the appropriate electrode response. In fact, disassembly of the electrode from the

2021326145

apparatus and scrubbing of the electrode was required to reestablish the appropriate response.

2. **Plans:** Continue to evaluate $\text{Ca}(\text{OH})_2$ and alternate base treated fillers for desired pH and stability over time. Complete KCl study and evaluate pH apparatus with aerosol generator. If identified, investigate other potential alternatives to the present "smoke pH" measurement procedure. Results of analytical testing will be compared with subjective and evoked potential results to evaluate interrelationships that might be used to predict results and indicate directions for future models.
 3. **Contributors:** CRD, BCR, ARD, CTSD, FTD.
 4. **References:** Southwick, R. Notebook No. 9178, pp. 34, 40 and 94.
- C. **Strategy:** Evaluate the effects of increasing filler and smoke "pH" on the sensory and subjective characteristics of cigarettes.
1. **Results:** The laboratory equipment for Sensory Physiology has arrived at INBIFO and is currently being tested for proper operation.
 2. **Plans:** Prepare the laboratory space for Sensory Physiology and install the instruments. Develop a nicotine/EP curve employing cigarettes at constant tar and varying nicotine levels. Conduct subjective and sensory evoked potential evaluations of cigarettes prepared from filler treated with $\text{Ca}(\text{OH})_2$ and alternate bases.
 3. **Contributors:** INBIFO

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**OTHER
PROGRAMS**

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PROGRAM NAME : Summary Other Programs
 WRITTEN BY : P. N. Gauvin
 PERIOD COVERED : Fourth Quarter, 1992

Coordinator Summary: The in-house development of biosensor technology to determine the concentration of gas phase compounds has continued through the fourth quarter, 1992. Piezoelectric crystal sensors coated with HA4 (nicotine specific) and anti-PMT antibodies plus lipid coatings such as phosphatidyl chrome, dioleoyl glycerol, and squalene were exposed to the head space of various gas samples. The phosphatidyl choline coated crystals responded well to the presence of all head space volatiles tested. Other lipid coated crystals responded more selectively.

A commercial sensor system was obtained which allows the exposure and measurement of response from a single Taguchi tin oxide gas sensor. The commercial sensor was used to examine the interaction of several types of Taguchi sensors, each of which is sensitive to a different class of compound.

In the area of computing systems, technical support and integration has been provided for desk-top computing devices in use within R&D. The system management database has been completed and is in use. All PC and Macintosh systems' hardware and software configurations, as currently known, have been loaded.

A neural network has been developed to predict the probability of switching as a function of smoker demographics and pre-switch brand attributes. Several types of multivariant calibration techniques were evaluated for their applicability to blend estimation of individual tobacco rods, including multiple linear regression, principal component regression, and neural networks. A project to replace the Semiworks HP1000 system with a DEC Micro Vax System was completed. The purpose was to provide production and engineering reporting functions that equal or exceed those found in the HP1000 system.

The ISO25 team continues to develop a quality manual for the combined CTSD/Product Audit Division. Documentation for the determination of cigarette circumference using the automatic Filtrona circumference gauge has been developed. The ISO Team is writing a standard operating procedure for this analysis.

Under Process Monitoring, a large-scale feasibility study was completed using the Katrina Protronics 112 Short Wavelength Near Infrared Transmission System for determining menthol-on-filler-on-line. The results of this study showed this technique was not applicable for a typical level of menthol application. Effort is continuing to provide NPP personnel with a rapid method for tobacco blend analysis. An Oriental/sheet study has been completed to determine the moisture and time at which non-damaged Oriental/selected sheet materials (RCB and RLTC) develop visible mold.

In an effort to develop vibration analysis as a tool for predicting and diagnosing machine health, a packer sub-assembly was obtained from York Engineering and set up in the Vibration Analysis Laboratory. The vibration signatures generated by the Geneva and cam-follower mechanisms are being analyzed to generate baseline information for future work.

In support of QA and Purchasing in implementing inspection of print web on the printing press, the feasibility of using Time Domain Integration (TDI) technology for image acquisition of printed web was demonstrated on a press at J. W. Fergusson. This approach was suggested to BOBST and Datacube (two companies in the image acquisition business). BOBST has developed an inspection system to be installed at Westvaco in January, 1993 for testing.

In the area of research on analytical methods, an informal collaborating study between PM-USA and FTR was conducted to evaluate the HPLC procedure as an alternate method for determining MH-30 (a tobacco sucker-inhibiting agent) in tobacco. The initiation of an on-line HPLC-MS/MS has been accomplished using the JEOL four-sector tandem mass spectrometer in order to develop and apply on-line HPLC tandem mass spectrometry techniques to the structural analysis of components in mixtures. In the past year a Varian unity spectrometer has been installed which has substantially enhanced the R&D capability for observing NMR in solids.

2021326149

PROGRAM NAME : Biochemical Special Investigations
 WRITTEN BY : M. Steele
 PERIOD COVERED : Fourth Quarter, 1992

BIOSENSOR TECHNOLOGY

Objective: Develop biosensor technology in-house to determine the concentration of gas phase compounds of interest. (B. D. Davies)

Piezoelectric Crystal sensors

Status: Piezoelectric quartz crystals coated with HA4 (nicotine specific) and anti-PMT (control) antibodies were exposed to the head space of gas sampling bags containing 5 ml of pyridine. Neither crystal changed frequency, indicating that pyridine adhered to neither the crystals nor the antibodies attached to the crystals. Similarly, when crystals coated with the HA4 and anti-PMT antibodies were incubated in a chamber with pyridine for 2 hours, no change in mass was detected on either crystal. A 2 hour exposure of these crystals to nicotine resulted in a frequency change in both crystals. The response of the HA4 crystal was much higher than the control anti-PMT crystal.

The 11 socket piezoelectric crystal multisensor array designed and built by Hugh McCafferty (R&D Engineering Development) was used to detect the presence of acetone, ethanol, or synthetic menthol. In these experiments, 4 uncoated crystals and 7 coated crystals were used. The lipid coatings used were phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), dioleoyl glycerol (DG), phosphatidyl inositol (PI), squalene (Sq), and stearic acid (SA). These lipids have been cited in the literature as effective in interacting with many volatile compounds.

Phosphatidyl choline coated crystals respond exceptionally well to the presence of all head space volatiles tested. Other lipid coated crystals respond more selectively. DG coated crystals respond well to the presence of acetone, but not the presence of menthol or ethanol. Squalene coated crystals respond well to the presence of menthol, but not to the presence of acetone. Squalene responds weakly to the presence of ethanol. Ethanol is readily detected by PE coated crystals. PE coated crystals respond only weakly to the presence of menthol or acetone.

Plans: Continue to test the HA4 and anti-PMT coated crystals in the presence of pyrrolidine using different parameters.

Continue efforts to determine if the antibody exposed to nicotine is a specific reaction (i.e., test the reaction with N-methylanabasine and normicotine, two compounds to which the HA4 antibody does and does not bind, respectively).

Determine the concentration of nicotine exposed to antibody coated crystals.

Investigate the response of the multisensor piezoelectric sensor array to simple mixtures of ethanol, menthol, and acetone as a model system. Also, begin to investigate the response of the multisensor array to the presence of different tobaccos.

References: Morrisette, E. C. Notebook 9176 pp. 191-192, 193-197; Notebook 9217 pp. 1-4, 10-13, 25-28.

Steele, M. Notebook 9202, pp. 40-100.

2021326150

Tin Oxide sensors

Status: A commercial sensor system was obtained from Figaro, U.S.A. which allows for the exposure and measurement of response from a single Taguchi Gas Sensor (TGS). The device was used to examine the interaction of several types of TGS, each of which is sensitive to a different class of compound, with various gases. For each analysis, a small aliquot (approximately 1 ml) of the head space above a sealed vial containing either ethanol, methanol, acetone, menthol or water was injected into a 5 liter (1 to 5000 dilution) sealed box containing a small circulation fan and one sensor. Immediately following injection, measurements were taken from the sensor, reflecting the interaction of the gas with the sensor. To date, all 7 different types of sensors have interacted strongly and differentially with the head space from methanol, ethanol, and acetone. The response to acetone has been the strongest. None of the sensors has responded to the head space sample above water. Only four of the sensors have responded to the head space gases above menthol. In addition, all of the sensors have responded to the head space above a tobacco sample placed in the sample chamber.

Plans: Work with R&D Developmental Engineering Personnel to develop a multisensor TGS based system. Work with CAD to develop the data analysis techniques to allow differentiation of individual gas phase compounds in complex mixtures.

References: Davies, B. Notebook 9218 pp. 1-5.

2021326151

PROGRAM NAME : CTSD ISO 25 Accreditation
WRITTEN BY : J. M. Garman
PERIOD COVERED : Fourth Quarter, 1992

Objective: To pursue and obtain ISO Guide 25 accreditation for the consolidated CTSD/Product Audit laboratory.

Status: The ISO 25 team continues to develop a quality manual for the combined CTSD/Product Audit division. A project team was chartered and has completed developing documentation for the determination of cigarette circumference using the automatic Filtrona circumference gauge. As a result, the Standards Lab has drafted PPI 640-118 and the ISO team is currently writing a standard operating procedure(SOP) for this analysis. Another team has been chartered and is currently developing documentation for the paper permeability analysis. The ISO team has completed a draft of a SOP for document control within the quality system.

Plans: Continue development of a quality manual for the consolidated CTSD/Product Audit testing operation. Coordinate the development of appropriate documentation as outlined in ISO Guide 25.

2021326152

PROGRAM NAME : Research on Analytical Methods
WRITTEN BY : S. Yang and N. Jensen
PERIOD COVERED : Fourth Quarter, 1992

I. Objective: To investigate the fate of propyl-paraben (PP) after being premixed with Avicel and applied on paper. Is it possible that PP might be (i) "entrapped" in the cellulose structure of Avicel and unextractable for quantitation? or (ii) hydrolyzed to p-hydroxy-benzoic acid? (Contributors: S. S. Yang and I. Smetena)

A. Status: Propyl paraben (PP) premixed with 6% Avicel solution at 0.05% level was applied to cigarette paper. These paper samples were analyzed repeatedly over 2 months using an HPLC procedure to determine the amount of propyl paraben present. Chromatographic separation was performed on a Hypersil ODS column with a mixture of methanol-water (75/25, v/v) as the mobile phase and UV detection at 254 nm. For paper samples containing 25-60 ppm, PP was slightly reduced in some samples over the period of 2 months. However, no significant loss of PP was observed in the paper samples applied with 10 ppm of PP. Since the assumption was made that PP might be "entrapped" in the layer formed by Avicel, it was important to notice any change of Avicel amount vs. PP reduction. No conclusion about the entrapment of PP should be made before substantial data are collected. The possibility of hydrolysis of propyl paraben to p-hydroxy-benzoic acid (HBA) also was studied. Some aged samples, in which PP almost disappeared after being stored for more than 8 months, along with the above two-month-old samples were analyzed and no HBA was found. A more vigorous test was conducted by mixing 10% HCl and 10% NaOH separately with a standard solution of PP (100 µg/mL) followed by shaking overnight. No evidence of hydrolysis was observed in either case.

II. Objective: An informal collaborative study between PM-USA and FTR was conducted to evaluate the HPLC procedure (by S. S. Yang, PM USA) as an alternative method for the determination of MH-30 in tobacco. (Contributors: S. S. Yang and I. Smetena)

A. Status: Seventeen ground tobacco samples provided by FTR were analyzed in labs of PM-USA and FTR using three different methods, including the HPLC method and two colorimetric methods as shown below.

PM USA: HPLC Method (Yang, J. Chromatogr., 595, 1992, p. 346), Colorimetric method (NCSU, North Carolina State Univ., S.A. Meyer and T.J. Sheets, Rev. Environ. Contam. Toxicol., 98, 1987, p. 43)

FTR: HPLC Method (Yang), Colorimetric Method (ISO, International Organization for Standardization, ISO Method No. 4867).

The data obtained from this study show that our HPLC method can generate reasonably accurate results consistent with the results from the other established methods.

1. Plans: Continue to use the micellar HPLC method (MH determination) developed in ARD for research or analytical support as needed.

III. **Objective:** To determine if there is any anethole on competitors' products. (Contributors: S. S. Yang and I. Smetena)

A. **Status:** Sixteen packs of cigarettes, mostly from our competitors, were analyzed using an HPLC procedure (by S. S. Yang) for the presence of anethole. Each component of cigarettes (filters and filler with paper) was analyzed separately due to the fact that anethole migrates. Since anethole also migrates to packaging materials, a section of the aluminum foil, approximately 0.16 grams (the part of foil covering the top of filters), also was analyzed to further measure the migration of anethole. The results show that some of the commercial cigarettes from the marketplace contain anethole, e.g., Winston Lights (0.38 $\mu\text{g/cigt}$), Camel Lights (0.48 $\mu\text{g/cigt}$), Monarch Regular (0.80 $\mu\text{g/cigt}$) and Monarch Lights (0.69 $\mu\text{g/cigt}$). The detection limit for tobacco material is 10 ppb.

1. **Plans:** There are two studies still in progress. 1) Distribution of anethole in cigarette components and packaging material. 2) Determination of residual flavor in tobacco materials (e.g., RL, RCB, etc.) using anethole as a tracer.

IV. **Objective:** Develop a microscopic technique to evaluate density differences among RL sheets. (Contributors: L. Thompson and D. Miser)

A. **Status:** There was a need proposed by engineering to distinguish density differences among various RL sheets microscopically. It was felt that the best contrast would be obtained from a transmitted light source. A technique was developed in which a light box served as that source. The RL sheet was placed on top of the light box, brought into focus, and the transmitted image was color photographed. This produced a macroview of density differences which could be characterized as having dark and light areas. Other important differences observed were related surface uniformity or non-uniformity and the presence or absence of holes in the RL sheet.

1. **Plans:** This microscopic technique has been developed and is available for testing as warranted.

V. **Objective:** To develop and apply on-line high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) techniques to the structural analysis of components in mixtures. (Contributor: T. Sumpter)

A. **Status:** The initiation of on-line HPLC-MS/MS has been accomplished using the JEOL four-sector tandem mass spectrometer. This technique allows the selection (using MS1) of multiple specific components of mixtures for structural studies by MS/MS in real time as they elute from the HPLC column. The following capabilities have been demonstrated in this laboratory:

- sequential precursor ion selection, in which several components from a single HPLC run can be selected for MS/MS analysis.
- rapid scan speeds, allowing averaging of multiple MS/MS spectra obtained for components exhibiting narrow peak widths obtained from capillary HPLC.
- analysis of both large and small molecules, ranging from ca. 190–750 amu. These values by no means represent the mass limits of this technique.

- HPLC-MS/MS analysis of positive and negative precursor ions, which allows for taking advantage of selectivities in ionization efficiencies.

This methodology has been applied to several problems. Negative ion MS/MS has been used to examine mixtures of sugars separated by analytical HPLC (3.9 mm i.d. column) with post-column addition of the fast atom bombardment (FAB) matrix. Capillary HPLC MS/MS has been applied in the sequencing of tryptic peptides at the 30 pmol level. Also, negative ion HPLC-MS/MS has been used in the investigation of an unknown component in flavor mixtures.

1. **Plans:** Develop collaborative interactions throughout R&D applying on-line HPLC-MS/MS methods to the structural characterization of components in mixtures.

VI. Objective:

To install and implement the use of the Cosa TN-05 Nitrogen Analyzer for total nitrogen analysis in aqueous matrices. (Contributor: W. Ryan)

- A. Status:** The Cosa Instrument Corporation TN-05 Nitrogen Analyzer, recently installed in the Chemical Analysis Section, is designed to measure total nitrogen in aqueous matrices by converting the chemically bound nitrogen to NO by high temperature vaporization/oxidation of the sample. The evolved NO subsequently reacts with ozone to form excited NO₂ which is detected by chemiluminescence.

Evaluation of the instrument is in progress, and initial work indicates an accuracy of 98% based on pure inorganic and organic compounds. Recovery studies with nicotinic acid added to the water extracts of various tobacco materials had an average of 92%. Studies using bright, burley, RCB and MT showed the system had a precision of 6 to 10% depending upon the matrix.

The current work has shown that the TN-05 provides rapid nitrogen analyses of water samples. However, it is apparent that the analysis will not be routine; each sample will require some knowledge of the matrix and the analysis will need to be modified and adjusted to accommodate the sample matrix. Further, it should be emphasized that this system is not an approved methodology for licensing requirements.

VII. Objective: Enhanced capability for NMR spectroscopy of solid materials using the new Unity 200 WB NMR Spectrometer. (Contributor: J. Wooten)

- A. Status:** The emphasis of NMR spectroscopy in recent years at Philip Morris has shifted from the determination of molecular structure to the characterization of materials. In this endeavor, the observation of NMR in solids has gained considerably in importance and has involved the introduction of new experimental techniques into our facility.

In the last year, a Varian Unity Spectrometer was acquired which substantially enhances the capability for observing NMR in solids. It utilizes a magnet with a large bore (80 mm) that can accommodate a large variety of probes. Included with the spectrometer is a probe with a 14 mm sample rotor size which requires the widebore magnet. The conventional rotor size is only 7mm, but the larger probe significantly increases the

2021326155

sensitivity in cases where there is ample sample available. The observation of carbon NMR involves the techniques called cross polarization and magic angle sample spinning (CPMAS NMR). The new probes are more RF efficient and spin faster than previous probes, affording spectra with improved resolution and sensitivity, free of interfering spinning sidebands. The RF components of the spectrometer deliver higher power with greater stability and control than was previously available. This provides versatility and reliability for performing the cross polarization experiment, and introduces enhanced capability for performing a variety of previously inaccessible RF pulse techniques such as "CRAMPS", the experiment for observing high resolution proton NMR spectra of solids. The spectrometer easily switches between observation of a variety of nuclei and has variable temperature capability. The spectrometer operation and data processing is substantially enhanced by a Sun workstation which also interfaces the spectrometer with the R&D local area computer network.

In its first year of operation, the applications and types of materials that have been examined have been varied and include the following: ^{13}C characterization of hydromagnesite paper fillers, ^1H , ^{31}P , ^{13}C characterization of papers sized with monopotassium phosphate, measurement of vanillin in derivatized cellulose, ^{119}Sn NMR of carbon monoxide catalysts, determination of cellulose crystallinity in wood pulp papers, determination of the effect on molecular mobility in tobacco due to tobacco processing such as steam tunnel treatment, measurement of pectin in blended leaf, comparison of the composition of Japanese and domestic tobaccos, characterization of polyphenolics in exhaustively extracted plant tissue, and characterization of immobilized nicotine, solid flavors and flavor release agents. The number of applications is expected to expand as the usefulness of the technique becomes generally recognized. Potentially, any of the solid materials associated with cigarettes are amenable to analysis such as tobacco, re-constituted tobaccos, paper and paper additives, adhesives, filter materials.

2021326156

PROGRAM NAME : Process Monitoring
WRITTEN BY : R. Kanipe
PERIOD COVERED : Fourth Quarter, 1992

I. Objective: To evaluate an at-line sugar monitor to test for uniformity of burley spray application. (M. Parrish)

A. Status: Although the TM55 sugar monitor will not be used to measure absolute levels of the sugar content on burley strip, an evaluation was completed to ensure that the instrument response is linear over the anticipated range and that the responses are in some reasonable agreement with laboratory data. The results showed that the factory set span and zero are sufficient for this application. The instrument response is linear over the range of 1 to 15% and is within 2% (absolute) of the laboratory values. Grab samples of cased and uncased burley were collected from the conventional burley spray cylinder on October 22nd. One hundred cased and twenty-five uncased strips of sufficient size (2"x2") were randomly selected. Sample analysis is in progress. J. Gear continues to assess the capability of the method to statistically compare the old and new processing techniques.

1. Plans: To complete the small scale study and support J. Gear's efforts.

II. Objective: To develop and implement an on-line monitor for menthol-on-filler. (W. Lewis)

A. Status: A large scale feasibility study was completed using the Katrina Protronics 112 short wavelength near infrared transmission system for determining menthol on filler on-line. The results of the study showed that this technique was not applicable for typical levels of menthol application (<1%). As this exhausted the available options for on-line determination, efforts shifted to an at-line approach using a thermal treatment to isolate the menthol for analysis. This technique would require automatic sampling, followed by an automated at-line analysis. Although technically feasible, implementation in a manufacturing environment would be problematic. As a result, further active investigations for this application have been discontinued.

1. Plans: To keep abreast of new technology or enhancements to current on-line technology which may be applicable.

III. Objective: To develop and implement a rapid method for tobacco blend analysis. (C. Harward)

A. Status: FTIR and XRF analyses of six grindings of the Bokelman sample set are nearly completed. These data will be used to develop a preliminary calibration for blend composition. Early evaluations using PLS indicate that all components can be quantified to some level of precision. J. Blankinship is providing assistance in defining the wavelength regions which are most unique in characterizing the various components. His efforts should significantly reduce data processing time for calibration. The standard deviation will be used to measure the blend uniformity.

2021326157

1. **Plans:** To provide NPP personnel with the estimated level of measurement precision for each of the eight blend components based on the Bokelman sample set. To establish a calibration using the BRICA blend components. To continue to refine the procedure to achieve the highest possible precision.

IV. Objective: To provide support to the NET moisture monitors.

- A. **Status:** A replacement board for sensor (A) was installed to correct the instability problems detected using the Cal Check Standards, and the instrument has been installed on-line by the NET engineers. Placement of sensor (A) on-line makes sampling for calibration or use of the cal checks impossible. NET personnel have been informed of the problem; modifications are planned to make this sensor accessible.

1. **Plans:** To provide support for calibration adjustment on-line.

V. Objective: To evaluate the reference method used for the calibration of on-line moisture monitors. (G. Hicks-White and B. Kanipe)

- A. **Status:** Alternative algorithms are being investigated in collaboration with Moisture Systems to address the "burley effect". Installation in the Semiworks has been postponed until the evaluation of the alternative algorithms is completed. D. Leyden and M. Crumly (PM Industrial Engineering) visited Moisture Systems on October 27th to discuss the costs associated with implementation. T. Fletcher of the Standards Lab is surveying the actual number of moisture monitors that are actively being utilized.

Refinements to the GC water procedure are in progress. Microwave treatment is being pursued as an alternative to shaking in order to significantly decrease the extraction time from the current four hours. Preliminary evaluations using this technique are favorable.

1. **Plans:** To continue to test and refine new algorithms and evaluate the effects of casings and expanded products. To continue studies to improve the GC water extraction time.

VI. Objective: To determine the moisture and time at which non-damaged oriental and selected sheet materials (RCB and RLTC) develop visible mold.

- A. **Status:** The oriental/sheet study was completed and documented.

1. **Plans:** To issue a completion report summarizing the three lab studies (bright, burley, and oriental/sheet).

2021326158

PROGRAM NAME : Computer Applications Division
WRITTEN BY : B. Kane, P. Lipford, J. Blankinship, J. Palesis, J. Stimler,
M. F. Allred, Jr. and A. L. Lewis
PERIOD COVERED : Fourth Quarter, 1992

I. Objective: Desktop and Network Support. Provide planning, technical support, and integration for the desktop computing devices in use within the Research Center.

A. Status: The System Management Database has been completed and is in use. All PC and Macintosh systems' hardware and software configurations, as we currently know them, have been loaded. This database has replaced all previous tracking methods for R&D computer system configurations. In addition to the database efforts, procedures have been defined and a form has been generated to insure that the database is kept current as changes to these systems occur.

It has been decided, for both budgetary and technical reasons, to put the PC backup project on hold. We have plans to evaluate and implement a general purpose network based backup procedure for all R&D computer systems in 1993. Our hope is that this overall backup strategy will supply the PC backup capability.

A pilot of the 800-number desktop software support has been initiated. Approximately 50 users have been utilizing the 800-number since mid-October. As of this time, no major complaints about the service have been registered with CAD.

An IS network connection for Macintosh systems has been installed. The four applications requested for this connection have been installed and tested. As of this time, the only shortcoming appears to be data transfer speeds. This was anticipated but had to be accepted in order to provide access in a timely manner without sacrificing security.

1. Plans: Load the remaining R&D computer systems' configuration information. These systems include the SUN, DEC and IBM systems.

Complete the 800-number pilot, accumulate comments and opinions from those who participated and make any needed adjustments. Once this is complete initiate R&D wide usage of the telephone support.

Install PC security software on all appropriate R&D PCs. At this time lab based data collection PC's that need to run unattended will probably be excluded. Unless unknown problems arise we expect to cover all office-based PCs.

II. Objective: Data Storage Management. Provide economies of scale and common availability of server hosts services for R&D computing requirements. Provide and manage shared computer resources. Integrate the heterogeneous computing environment to minimize inter-vendor incompatibilities.

A. Status: Phase III of the DECsystem 5900 Installation/Transition Plan (provide vendor and system software suites comparable to the existing Vax/8650 systems) was completed on August 31, 1992. The physical de-installation of the two Vax/8650 systems was completed in September, 1992. The Remaining Vax/8650 system has been reconfigured and is available to the user community with a node name of pm700.

2021326159

A more comprehensive and cost effective solution for data storage and management is required to maintain data integrity and acceptable production capacity for the R&D computing environment. The initial evaluation of two Hierarchical Storage Management Systems (HSM) for our computing environment began this reporting period. Particular attention is being given the existing timesharing and distributed computing environments to ensure efficient storage management.

Hardware upgrades for five Sun Server systems have been purchased and will be installed and configured when received. These upgrades will provide more resources, services, and capacity for the support of the distributed computing environment.

1. **Plans:** Complete HSM evaluation and make recommendation to management. Complete installation and configuration of the server system upgrades.

III. Objective: Analysis and Modeling of Brand Switching Behavior. Analyze consumer data to discover general relationships between brand switching behavior vs. smoker demographics and product attributes. Develop neural network models which can be used to predict brand switching behavior as a function of smoker demographics and product attributes. Explore the use of bi-linear regression techniques such as Principal Component Regression (PCR) and Partial Least Squares (PLS) to help predict and interpret brand switching behavior.

A. Status: A neural network model was developed to predict the probability or likelihood of switching as a function of smoker demographics and pre-switch brand attributes. For this purpose, a training set of over 60,000 examples was constructed from the 1990-1991 Tracking study. The explanatory variables for this model are the demographics (region, age, race, sex, Nielsen county, education, income, and marital status) of the smoker, the attributes (length, menthol type, packing, tipping color, price, and tar level) of the smoker's brand, and possibly the brand family. Models were developed both with and without brand family to determine whether it improves the predictive performance of the model. The predictive performance of the model was slightly better when the pre-switch brand family was included as an explanatory variable.

A separate neural network model was developed to predict the probability of switching as a function of only the pre-switch brand attributes and the pre-switch brand family - i.e. no smoker demographics.

Data from the 1990-1991 Tracking study were statistically analyzed using BMDP to determine general relationships between the probability of switching vs. smoker demographics and pre-switch brand attributes. The following factors were determined to have a significant impact on the likelihood of switching: age, race, gender, income, education, Nielsen county (degree of urbanization), cigarette length, and packing type. A report is being prepared to document the results of the study.

A set of neural network models was also developed to predict the post-switch brand attributes as a function of the pre-switch brand attributes and the pre-switch brand family. Separate neural networks were developed for each post-switch brand attribute (length, menthol type, packing, tipping color, price and tar level). A set of neural networks are also being developed to predict the post-switch brand family as a function

2021326160

of the pre-switch brand attributes and the pre-switch brand family. These and the previously mentioned neural networks are being incorporated into a brand switching model for potential use in consumer research, direct marketing, brand management, and product design.

1. **Plans:** Present principal findings to the Product Evaluation Division and prepare a research report. Develop a set of neural network models to predict brand switching behavior as a function of smoker demographics only. Develop a decision support system for user operation of these models. Explore the use of PCR and PLS to help predict and interpret brand switching behavior.

IV. Objective: Blend Analysis. Develop techniques to estimate, classify, and recognize the composition of a tobacco blend given a set of observed spectroscopic and other analytical properties. Also, determine which analytical properties provide the most information about blend composition.

- A. **Status:** Several types of multivariate calibration techniques were evaluated for their applicability to blend estimation of individual tobacco rods – including multiple linear regression, Principal Component Regression, Partial Least Squares (PLS) regression, linear mixture models, and neural networks. A research report is being written which summarizes these calibration techniques.

Software techniques were developed to compute least-square maximum likelihood blend estimates for diffuse NIR reflectance spectra. These techniques use the linear mixture model with matrix inversion as well as the simplex optimization method to compute the blend composition which best fits the observed property vector. These techniques also provided ARD with a set of wavelengths which have the highest impact on the estimate of the blend components.

A software algorithm was developed for greatly reducing the number of wavelengths (channels) prior to the use of PLS regression. The algorithm is based on an article in the Journal of Chemometrics titled "Wavelength Selection in Multicomponent Near-Infrared Calibration." This procedure will result in an improvement in predictive accuracy as well as a tremendous decrease in processing time. The algorithm was applied to NIR and X-ray fluorescence data from ARD to determine a subset of channels which are most correlated to the concentration of a blend component with minimal interference from other wavelengths.

1. **Plans:** Continue application of the channel selection algorithm to analytical data from ARD. Test other channel selection algorithms based on the least square maximum likelihood technique. Complete a research report on multivariate calibration techniques. Develop PLS software for blend estimation of individual tobacco rods.

V. Objective: BL Dryer Belt Tracking. Use expert systems, fuzzy logic, and machine learning to improve tracking of the BL Dryer Belt and thus substantially increase production. Improved tracking will increase production by minimizing downtime, minimizing wear and tear of critical process components, and making it possible to expand the width of the belt which carries the slurry through the dryer.

- A. Status:** The BL Belt Tracking Expert System has been running Line II on a 24-hour basis since September 7, 1992. Tracking results indicate that, compared to PID control, the Expert System has substantially improved tracking under normal steady-state conditions and under major process disturbances. The measured benefits of these improvements include: (1) a 75 percent reduction in rubber roll wear and tear and (2) elimination of track-offs due to major process disturbances. The BL Plant estimates the potential benefit of these improvements, for all three lines, to be approximately \$350,000 per year.

One of the main problems still remaining with regard to BL Belt Tracking is start-ups. Starting up the belt manually, especially after track-offs, is a time-consuming process which costs the BL Plant over \$200,000 worth of downtime per year. In view of the Expert System's success in steady-state tracking, it is estimated that a fully automatic start-up procedure under Expert System control would substantially reduce start-up time and save the BL Plant approximately \$100,000 worth of downtime per year. Furthermore, improved start-ups under expert system control would reduce belt wear and tear and, most importantly, would make it possible to move to a wider belt and thus increase BL Plant capacity by approximately 6 percent.

Start-ups can be divided into two categories: "easy" start-ups and "hard" start-ups. Easy start-ups are performed when the belt stops in a fully aligned position. Hard start-ups are performed after the belt tracks off (i.e., hits the limit switch). Hard start-ups, on the other hand, must be performed partly manually: the belt must first be aligned by the operator before it can be started automatically under expert system or PID control. Thus, to achieve a fully automatic start-up the belt alignment process must also be automated. A fully automatic start-up would enable operators to handle both easy and hard start-ups by simply pressing the start button.

An expert system capability to handle easy start-ups has already been developed and is presently being optimized.

- 1. Plans:** Optimize the easy start-up feature and then develop a hard start-up capability.

- VI. Objective:** Replace the Semiworks HP 1000 system with a DEC Micro VAX system. Provide production and engineering reporting functions that equal or exceed those found in the HP 1000 system.

- A. Status:** This project is closed. An Operator's Guide for the new system was delivered. The DEC 2150 laser printer, which was purchased for compatibility with the Fisher-Provox programs as well as with RS/1 and VMS spooling facilities, is working well.

- 1. Plans:** Provide additional documentation consisting of a Troubleshooter's Guide and a Programmer's Reference. Provide support as needed.

- VII. Objective:** Capture information from three Cast Leaf lab instruments, transfer the information to the UNIX host and integrate it into a host database.

- A. Status:** The programs to capture data from the three instruments were developed and tested. Documentation was prepared.

1. **Plans:** Depending on availability of database personnel, finalize the database load procedure.

2021326163

PROGRAM NAME : Print Web Inspection
WRITTEN BY : K. H. Shafer
PERIOD COVERED : Fourth Quarter, 1992

I. **Objective:** Support QA and Purchasing in the implementation of global inspection of print web on the printing press.

A. **Status:** The feasibility of using time domain integration (TDI) technology for image acquisition of printed web was demonstrated on a press in a joint effort by the departments of Engineering, Quality Assurance, Purchasing, and R&D at J. W. Fergusson in the first quarter. This approach was then suggested to Bobst and Datacube (two companies in the image acquisition business). Bobst has since developed an inspection system which will be installed in January at Westvaco for testing. Engineering, R&D, QA, and Westvaco have assessed the system capabilities by attending a demonstration of their prototype system held at Bobst in Peabody, Massachusetts on November 6. The single camera system is equipped with high frequency lighting and has a resolution of 25 mil over approximately 45 inches using a 2048 pixel TDI camera with 32 line integration. The system was operated at a speed of 690 ft/sec.

Westvaco has also evaluated and purchased a Futec system (independently evaluated by the R&D effort as being the only commercially viable technology for gross defect detection) which is to be installed in March.

1. **Plans:** A report will be issued documenting the R&D technology development of on-line web inspection.

2021326164

PROGRAM NAME : Predictive Maintenance
WRITTEN BY : K. H. Shafer
PERIOD COVERED : Fourth Quarter, 1992

I. Objective: Develop vibration analysis as a tool for predicting and diagnosing machine health and as a design aid for improving machine performance.

A. Status: In preparation of monitoring the vibration signature of an induced failure, a packer sub-assembly was obtained from York Engineering and set up in the vibration analysis laboratory. The vibration signatures generated by the Geneva and cam-follower mechanisms are being analyzed to generate baseline information for future work.

The fabrication of a newly designed portable shaker system has been completed for field testing of problem machinery. The development of a shaker system is part of an effort to apply more advanced vibration analysis and diagnostics techniques in the field.

The R&D predictive maintenance program will focus on the evaluation of the viability of vibration analysis for condition monitoring and diagnostics of packers as requested by Bob Hassell, Director of York Engineering, in a meeting held on October 14. A proposal of the 1993 project plan to accomplish this goal was reviewed at York Engineering on November 4 and accepted with minor revision. The effort is being coordinated with York Engineering, the Manufacturing Center (MC) and R&D.

1. Plans: Hardware and software needed to develop a database for analysis of packer signatures are being acquired so that signal processing work can be initiated. The installation of the accelerometers on packers at the MC will be completed by the end of the year.

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